A MILK-BORNE PARATYPHOID OUTBREAK IN AMES, IOWA *

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The frequency of paratyphoid fever in man is an unsettled question. Clinically the disease simulates true typhoid fever so closely that a diagnosis of the latter is almost always made; nor can the routine agglutination test with typhoid bacilli be relied upon to differentiate between the two diseases. Accurate statistics on the prevalence of paratyphoid infections are therefore lacking.

Conradi[1] found 18 paratyphoid cases among 235 patients apparently ill with typhoid, and Brion and Kayser,[2] in an investigation of 300 cases of fever during the two years, 1904-06, found 9 cases of paratyphoid infection. Seven of the latter were caused by B. paratyphosus B and 2 by B. paratyphosus A. In a study of 505 cases in Strassburg, Kayser[3] found 27 of the paratyphoid B, and 5 of the paratyphoid A type. According to Brown and Deeks,[4] 50% of apparent typhoid fever in the Canal Zone is really paratyphoid fever.

Literature on the epidemiology of paratyphoid fever is meager. Infected meat is generally regarded as an important means of spreading the disease. Several small outbreaks due to water and milk have been reported abroad. Fischer[5] reports 50 cases of paratyphoid which he attributes to a milk supply infected through 2 cows suffering from enteritis. In another outbreak, recorded by Nielsen,[6] the milk supply had been infected by a girl who nursed 2 paratyphoid patients in a neighboring town. In Graesvick, Germany, Gram[7] traced 16 cases to a milk supply and isolated the paratyphoid organism from river water which was used in the cow barn.

The outbreak which we are about to describe was small in extent, but since the causative organism was B. paratyphosus and milk the vehicle of infection we deem it of sufficient importance to be put on record.

THE PARATYPHOID OUTBREAK IN AMES

Ames is a typical college community with a population of 5000, exclusive of about 2700 students. It is situated on the main line of the Chicago and Northwestern railroad running east and west, and is the central point for several branch lines running north and south, as well as for the Interurban

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[1] Klin. Jahrb., 1907, 17, p. 351. Deutsch. Med. Wchnschr., 1904, 30, p. 1165.
[2] München. med. Wchnschr., 1902, 49, p. 611. Arch. f. klin. Med., 1905, 85, p. 525.
[3] Centralbl. f. Bakteriol., I, O., 1912, 40, p. 285.
[4] Cited, Kolle and Wassermann, Handb. d. pathogen. Mikroorganismen., 1913, 3, p. 1012.
[5] Klin. Jahrb., 1906, 15, p. 61.
[6] Tidsskr. f. d. norske Laegefor., 1907, 27, p. 333.
[7] Centralbl. f. Bakteriol., R., 1910, 48, p. 171.

railway, which communicates with a large number of outlying municipalities throughout the state. The location of the city, coupled with its migratory population, makes it an important focus for the spread of an epidemic.

Typhoid fever is not a reportable disease in the state of Iowa. Consequently, altho cases had been present in the city of Ames for several months, the health authorities were not aware of the fact. The occurrence of several cases in the family of one of the members of the faculty brought the matter to the attention of the college authorities. On November 14, this laboratory was authorized by the state board of health to undertake a complete study of the outbreak.

A canvass among the physicians revealed that, during July and August, 4 cases had been reported, none in September and October, and 11 between November 1 and 16. The four cases first mentioned were apparently not interrelated. Two were traced to out-of-town sources; the sources of the other two could not be determined. One of these may have been the focus of infection in the November outbreak, and will be discussed more in detail. A table of the November cases follows:

TABLE 1

THE CASES OF PARATYPHOID FEVER DURING NOVEMBER

Case	Date of Taking to Bed	Number of Cases
1	November 1	1
2	November 4	1
3, 4	November 6	2
5	November 8	1
6, 7	November 9	2
8	November 10	1
9*	November 11	1*
10	November 12	1
11	November 16	1

* Infection came from out of town (Arkansas); it will not be included in this discussion.

Case 1 was that of an adult who spent considerable time traveling about the country. The infection was first diagnosed as pneumonia; agglutination tests with typhoid bacilli made on November 12 and 15 were negative. A test made on December 20 gave a marked positive reaction. It is very likely that this case was an out-of-town infection.

Cases 2, 3, and 4 gave a history of weakened resistance through continuous, or intermittent, illness throughout the summer. The date of first symptoms in these instances, therefore, could not be definitely determined.

Cases 10 and 11 were due to contact infection. The former was in a family in which 2 other cases (3, 4) occurred on November 6. The latter (11) was in intimate contact with Case 6 during the prodromal period, and for at least 2 days after definite onset. Both these patients were inoculated with typhoid vaccine several days before coming down.

From the foregoing considerations it seems apparent that the actual period of causation was extremely short, perhaps not more than a day or two in the proximity of October 25. There seems to have been a prodromal period of 4 or 5 days.

Possible Sources of Infection other than Milk.—A large majority of the patients obtained their fruits and raw vegetables from the same dealer, but relatively few gave a history of eating the same kinds of raw vegetables.

Furthermore, this dealer supplies a large part of the community throughout the city, so that if these raw products were responsible for the outbreak, the cases ought to have been scattered throughout the city. In this outbreak all the cases were in a small section of the town. A study of the distribution of the cases as to ice cream consumption eliminated this food as a probable cause. The season of the year served to eliminate flies and insects.

The city water supply, obtained from 2 well-constructed, deep wells, is of excellent quality bacteriologically. Nine patients used city water exclusively. The tenth obtained water from a private well, but had access to city water at school. The extreme localization of the cases coupled with the wide distribution of the public water supply removes suspicion from this source.

Milk, the Probable Source of Infection.—Of the 10 patients 9 obtained milk exclusively from one dealer "D" during the causation period and up to the time of this investigation. The tenth had some milk from this dealer, but the infection was probably due to contact. Seven gave a history of drinking raw milk habitually, 2 had very little milk, and 1 had milk only with cereals for breakfast.

The distribution of the cases with reference to age points to milk rather than to water or other food substances as the vehicle of infection. Seventy percent of all the cases were among children under 14 years of age; or, if we omit the two cases due to contact, 62.5% were among children of milk-drinking age.

TABLE 2
THE DISTRIBUTION OF CASES OF PARATYPHOID FEVER ACCORDING TO AGE AND SEX

Age	Male	Female	Total	Percentage
0- 4	1	1	2	20
5- 9	1	1	2	20
10-14	1	2	3	30
25-29	...	1	1	10
30-34	...	1	1	10
45-49	1	...	1	10
Totals	4	6	10	100

The infected milk supply was obtained from a dozen farms in the vicinity of Ames. Careful investigation of these sources failed to disclose any typhoid-like case, and all evidence as to visitors and convalescents on these farms, as well as disease among the herds, was negative.

There were 2 possibilities for bottle infection. A case of apparent typhoid fever came down on July 28. On September 1, milkman "D" began to deliver milk to this patient. The patient did not leave her bed until October 15. From this date she gradually resumed her household duties. Such precautions as disinfecting stools and urine were discontinued, and about 3 weeks after her apparent recovery, her daughter came down with the fever (Case 7, Nov. 9). The possibility of bottle infection by this convalescent is evident.

On the other hand, there was opportunity for infection of the milk supply at the dairy. The milk dealer stated that he had had typhoid fever 4 years previous and that his wife and 4 children had all been down with the disease at that time (1910). An agglutination test (microscopic) with typhoid bacilli gave a questionable reaction with serum from the milk dealer's wife; bacteriologic examination of her feces showed the presence of paratyphoid-like organisms. This woman had charge of the washing and filling of the bottles, which were not sterilized, but merely rinsed in cold water.

The occurrence of all the primary cases in a restricted area of the community and on one milk route, the high percentage of infection among children, and the location of 2 possible sources of infection of the milk supply, are conclusive for the outbreak's having been milk-borne.

BACTERIOLOGIC FINDINGS

The proximity of the laboratory made it possible to use freshly collected blood samples for the bacteriologic diagnosis, thus being eliminated the possible errors of dilution in dried specimens. For diagnosis about 0.25 c.c. of blood was collected in a Wright capillary pipet, allowed to clot, and centrifugated. In the course of the routine agglutination tests with typhoid bacilli it was observed that agglutination of B. typhosus with a 1:40 dilution of the centrifugated serum was extremely slow (usually absent for an hour or more), while one specimen (Case 7) which was tested simultaneously against B. paratyphosus B brought about complete clumping of this organism in less than 15 minutes. Attention was therefore directed to the possible presence of some organism other than B. typhosus, and to the possibility that the outbreak was one of paratyphoid fever rather than true typhoid. Samples of urine and feces from 3 carrier suspects and 8 patients were examined for the presence of the causative organism, and macroscopic agglutination tests were made with B. typhosus and the A and B types of B. paratyphosus.

Isolation of Organisms.—The technic finally employed for the isolation of the causative organisms was as follows: Samples of feces and centrifugated urine were smeared directly onto Conradi-Drigalski plates; at the same time lactose bile tubes were inoculated with urine and feces, respectively, and after 4 to 5 hours' incubation at 37 C. new Conradi-Drigalski plates were made. All plates were incubated at the body temperature for 24 hours. If suspicious colonies developed, they were fished, streaked on fresh Conradi plates, and re-incubated. From this second series of plates several suspicious colonies (10 or 12) were fished onto agar. The cultures thus obtained were then inoculated into a series of sugars and various other confirmatory tests, to be described, were made.

In no instance did we obtain B. typhosus, while organisms of the paratyphosus type were secured in pure culture from the feces of a carrier suspect (the milk dealer's wife) and from the urine of 1 of the patients. On the Conradi plates, from the feces of 2 other patients, bluish-gray colonies were present which were identical in appearance

with those obtained from carrier and urine, but we did not succeed in separating them from B. coli. The cultural and morphologic characters of the isolated organisms are given in Table 3.

TABLE 3

CULTURAL AND MORPHOLOGIC CHARACTERS OF ORGANISMS OBTAINED FROM PARATYPHOID PATIENTS

Organism	M_2	R_4	R_3
Source	Feces of carrier suspect	Urine of Patient 8	Urine of Patient 8
Conradi-Drigalski plates	Colonies small, of a bluish-gray color with medium discolored slightly bluish		
Gram stain	—	—	—
Motility	+	+	+
Morphology	Short rod	Short rod	Short rod
Dextrose	+	+	+
Lactose	—	—	—
Saccharose	—	—	—
Maltose	±	±	±
Levulose	+	+	+
Galactose	+	+	+
Mannite	+	+	—
Raffinose	—	—	—
NO_3 reduced	+	+	+
Indol	+	—	+
Milk	Acid then alkaline	Acid	No change

From Table 3 it is evident that the isolated organisms belong to the paratyphoid group. It will be observed that indol was produced by 2 of the strains. The power to produce indol is not generally ascribed to B. paratyphosus, but Poppe[8] found that indol-production by B. paratyphosus was dependent upon the grade of peptone employed in the test, while Andrejew working with a series of strains of B. paratyphosus B repeatedly obtained positive indol reactions. One of us[9] has observed indol-production in paratyphoid cultures isolated from cholera-infected hogs.

The cultural tests were confirmed by the use of differential media. The materials used were: Loeffler's malachite-green dextrose nutrose

[8] Ztschr. f. Infektionskrankh. d. Haustiere, 1908-09, 5, p. 42. Ztschr. f. Immunitätsf., O., 1912, 13, p. 185.

[9] Eberson: Jour. Infect. Dis., 1915, 17, p. 331.

solution (Loeffler I); Loeffler's malachite-green lactose nutrose solution (Loeffler II); Barsiekow's litmus nutrose dextrose solution (Barsiekow I); Barsiekow's litmus nutrose lactose solution (Barsiekow II); Hetsch's litmus nutrose mannite solution; Petruschky's litmus whey; plain milk, dextrose broth, lactose broth; and orcein agar and neutral-red agar. The results are shown in Table 4.

TABLE 4

CULTURAL RESULTS WITH USE OF DIFFERENTIAL MEDIA

Strain	Loeffler		Barsiekow		Hetsch	Litmus Whey	Milk	Dextrose	Lactose	Orcein Agar	Neutral-red Agar
	I	II	I	II							
M 2	Coagulated	Unchanged	Coagulated; acid	Unchanged	Coagulated; acid; slight gas	Alkaline	Unchanged	Gas	No gas	Cleared	Fluorescence; gas
R 4	Coagulated	Unchanged	Coagulated; acid	Unchanged	Coagulated; acid; slight gas	Slightly acid	Unchanged	Gas	No gas	Cleared	Fluorescence; gas
Paratyphosus B	Coagulated	Unchanged	Coagulated; acid	Unchanged	Coagulated; acid; slight gas	Alkaline	Unchanged	Gas	No gas	Cleared	Fluorescence; gas

Agglutination Tests.—A rabbit was immunized against M_2 by repeated intravenous injections of an agar culture, which was suspended in salt solution and heated at 60 C. for 30 minutes. Ten days after the last injection the animal was bled and the serum tested macroscopically against the homologous strain, and against strains of B. paratyphosus A and B. It was found that a 1:1200 dilution of the serum agglutinated the homologous strain, but that a 1:100 dilution would not agglutinate B. paratyphosus A and B. Another rabbit was immunized against B. paratyphosus B and the serum tested against the organisms isolated. The tests were negative.

From the agglutination tests it is apparent that the serum specific for B. paratyphosus B did not agglutinate the isolated strains and vice versa; but this lack of confirmation of the cultural characters does not vitiate the conclusion that the organisms are of the paratyphoid group. We may cite Messerschmidt,[10] who isolated from a paratyphoid fever patient an organism which simulated B. paratyphosus B in all cultural characters, but which was not agglutinated by sera produced by any member of the typhoid-colon group. Under certain conditions organisms which are well agglutinated may become non-agglutinable. According to Paltauf,[11] certain strains are really composite, possessing both agglutinable and non-agglutinable members,

[10] Centralbl. f. Bakteriol., I, O., 1912, 66, p. 35.

[11] Kolle and Wassermann, Handb. d. pathogen. Mikroorganismen., 1913, 2, p. 502.

and the latter may predominate at times. The agglutination test is rather variable; many observers have recorded the isolation of strains which were but slightly agglutinable, if at all.

The conclusion we have reached, that this outbreak was due to the paratyphoid infection, was not based merely on the isolation of suspicious organisms, but primarily on tests obtained with the sera of the patients.

On December 20, about 6 to 7 weeks after the onset of the cases, blood samples were collected from 4 patients and tested macroscopically against B. typhosus, B. paratyphosus A, and B. paratyphosus B. The results are shown in Table 5.

TABLE 5

MACROSCOPIC AGGLUTINATION TESTS WITH TYPHOID BACILLI AND SERA FROM PATIENTS

Case	Organism	Dilution of Serum				
		1-100	1-200	1-500	1-800	1-1200
3	B. typhosus	—	—	—	—	—
	B. paratyphosus A	+	—	—	—	—
	B. paratyphosus B	+	+	+	—	—
11	B. typhosus	—	—	—	—	—
	B. paratyphosus A	+	—	—	—	—
	B. paratyphosus B	+	+	±	—	—
8	B. typhosus	—	—	—	—	—
	B. paratyphosus A	+	±	—	—	—
	B. paratyphosus B	+	+	±	—	—
1	B. typhosus	+	+	+	+	+
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	—	—	—	—	—
9 (a)	B. typhosus	—	—	+	+	+
	B. paratyphosus A	+	—	—	—	—
	B. paratyphosus B	+	—	—	—	—

Case 9(a) was not connected with the outbreak. This was a typical typhoid patient, who became infected while in Arkansas. The serum was employed as a control. Lack of agglutination in the low dilutions is probably due to the presence of pro-agglutinoids.

From Table 5 it appears that Case 1 is markedly different from the others. The epidemiologic evidence was also of a nature to throw doubt on the connection of this case with the outbreak. The patient was an adult about 50 years old, who spent considerable time traveling about the country. It is possible that infection in this instance occurred out of town.

The other three sera agglutinated B. paratyphosus B in higher dilutions than B. paratyphosus A, while B. typhosus was not clumped. The

sera were not very potent. The agglutinins reach a maximum in about 2 weeks after which there is a rapid decrease. In view of the fact that these tests were made about 6 to 7 weeks after the onset of the cases, more potent sera could hardly be expected.

Case 3 was in a home where 2 others were affected, while Case 11 was contracted through contact with Case 6. Earlier in the investigation one patient (7) agglutinated B. paratyphosus B much more rapidly than B. typhosus. Thus, it is quite conclusive that at least 7 patients were affected with paratyphoid fever.

SUMMARY AND CONCLUSIONS

For the following reasons we feel satisfied, from a study of the epidemiologic evidence, that milk was the vehicle of infection.

All the primary cases were on one milk route. Of the two cases attributed to contact, one used this milk exclusively, the other occasionally.

Sixty-two and five-tenths percent of the primary infection was among children, all under 14 years of age. This high percentage of infection is characteristic of milk-borne outbreaks.

There are 2 possible sources of infection of the suspected milk supply.

The age incidence and the localization of the cases eliminate other food supplies, such as water or vegetables.

From the bacteriologic study of the cases, it appears that after 6 to 7 weeks the patients' sera were more potent towards B. paratyphosus B than towards the A type, while the typhoid organism was not agglutinated. This, together with the atypical clinical course and diagnostic reactions of agglutination with typhoid bacilli leads us to regard the outbreak as one of paratyphoid fever.

Altho it is not possible definitely to ascertain whether the infection was from a carrier or a convalescent, there is no doubt that proper handling and bottling of the milk would have forestalled this outbreak. The two cases attributed to contact were both inoculated against typhoid several days before coming down. This was before the disease was recognized as paratyphoid fever. Immunization against paratyphoid might have protected these patients. This outbreak points out the necessity of testing questionable and negative typhoid specimens against the paratyphoid strains.